Aptamer-Bioconjugate Drug Delivery Device

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Cancer

- 40% of Canadians will receive a diagnosis of cancer over their lifetime (www.cancer.ca)
- Standard chemotherapy agents kill rapidly dividing cells
  - Cancer, hair follicles, gastrointestinal cells
  - Significant toxicity

→ Limited therapeutic use, particularly in advanced stage disease

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Advantages of Nanomaterials in Drug Delivery

1. Size

- Long Biological Half-life[1]
- Enhanced Permittivity and Retention Effect (EPR) [1]

2. Controlled Release

- Longer effectiveness of treatment[1]

3. Large Surface Area

- Targeting Ligands
- PEG, Contrast Agents

1. Wang AZ, Langer R and Farokhzad OC. "Nanoparticle delivery of cancer drugs". Annual Reviews of Medicine, 63 (2012) 185.

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Design Requirements

1. Biocompatible
2. Device uptake by cancer cells
3. Able to load drug
4. Controlled sustained release profile
5. Able to kill cancer cells with encapsulated drug
6. Can be produced reliably and give similar outcomes

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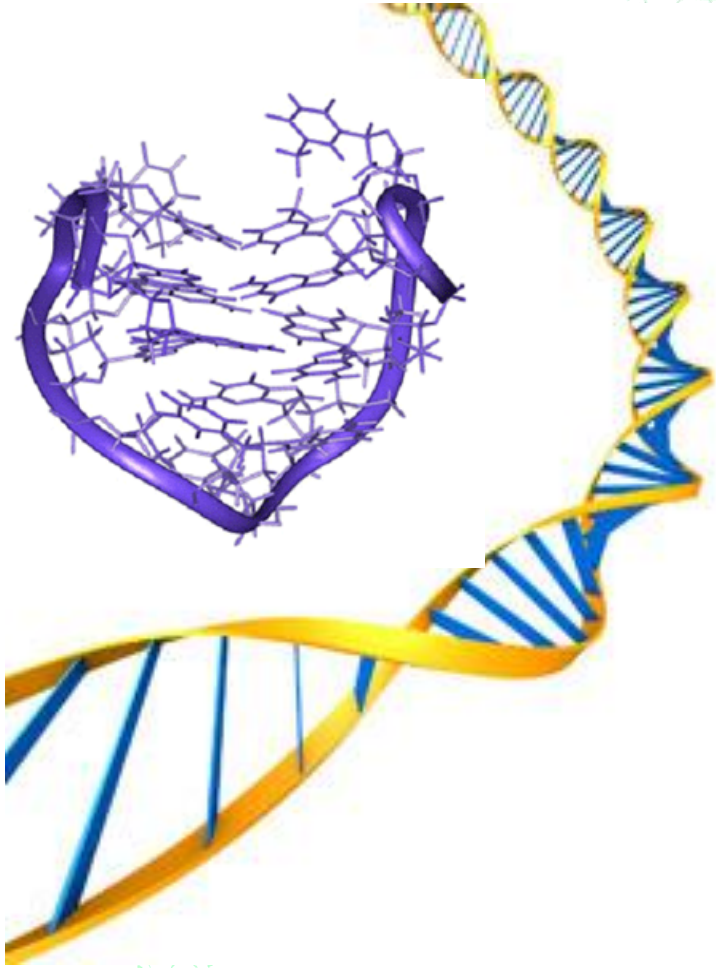
Our Design

Aptamer – “to fit”

- AS-1411 ssDNA oligomer of 22 nucleic units – in phase II clinical trials

- Binds to nucleolin receptor which is expressed by many cancer cells

- Poor biological lifetime if non-conjugated



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EcoSphere®

- Product of EcoSynthetix
- Cross-linked starch nanogel
- Uniform small size (120 nm diameter)

Doxorubicin & Docetaxel

- Commonly used chemotherapeutic agents

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Why Starch?

- Many other materials have been studied for drug delivery: PLGA-PEG; Liposomes; porous silica; gold nanoparticles
- EcoSphere starch nanoparticles can be reliably produced at large scale for low cost
- Starch is a food material – biocompatible
- Hydrophilic – results in lengthy biological lifetime
- Multitude of reactions to modify starch

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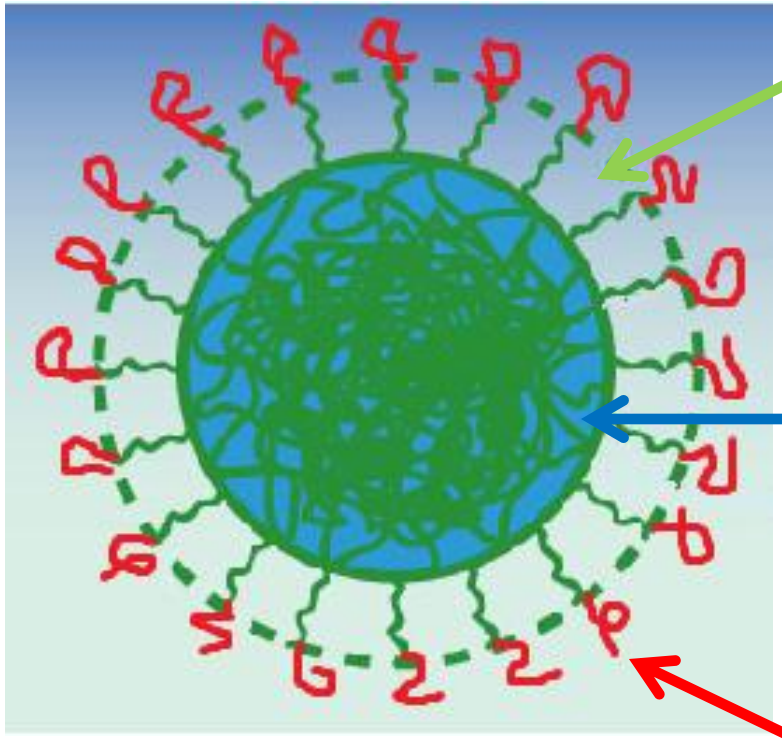
Drug Delivery Device (Patent Pending)



EcoSphere

- Can vary cross-linking density
- Negatively charged

Drug

- Loaded actively or passively
- 5% by-mass loading

Aptamer

- Covalently bound
- Attached using EDC/NHS reaction

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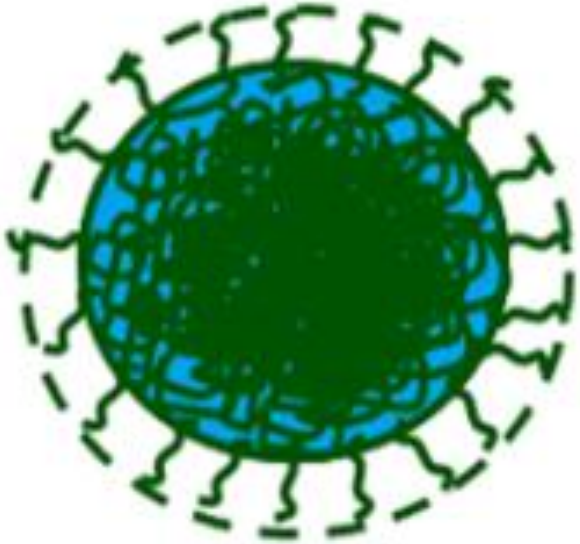
Fabrication Process

Based on [6] Mangalam et al.
"Cellulose/DNA hybrid nanomaterials"

Initial Nanoparticle

EcoSphere Starch Nanoparticle



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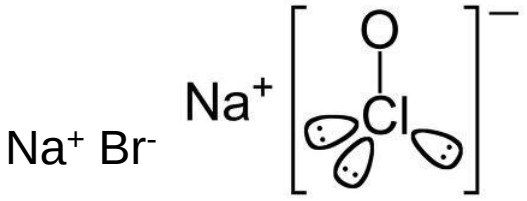
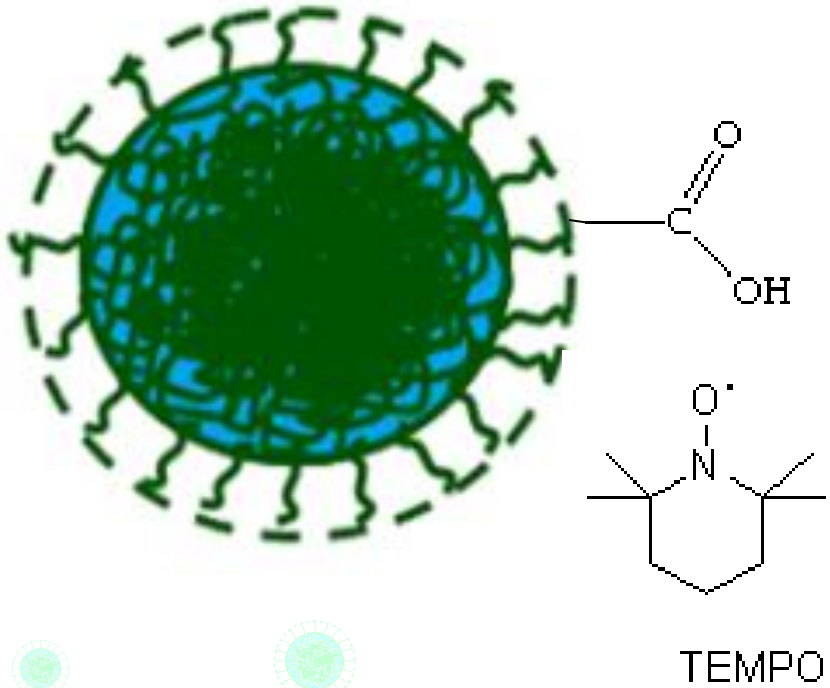
Tempo Oxidation





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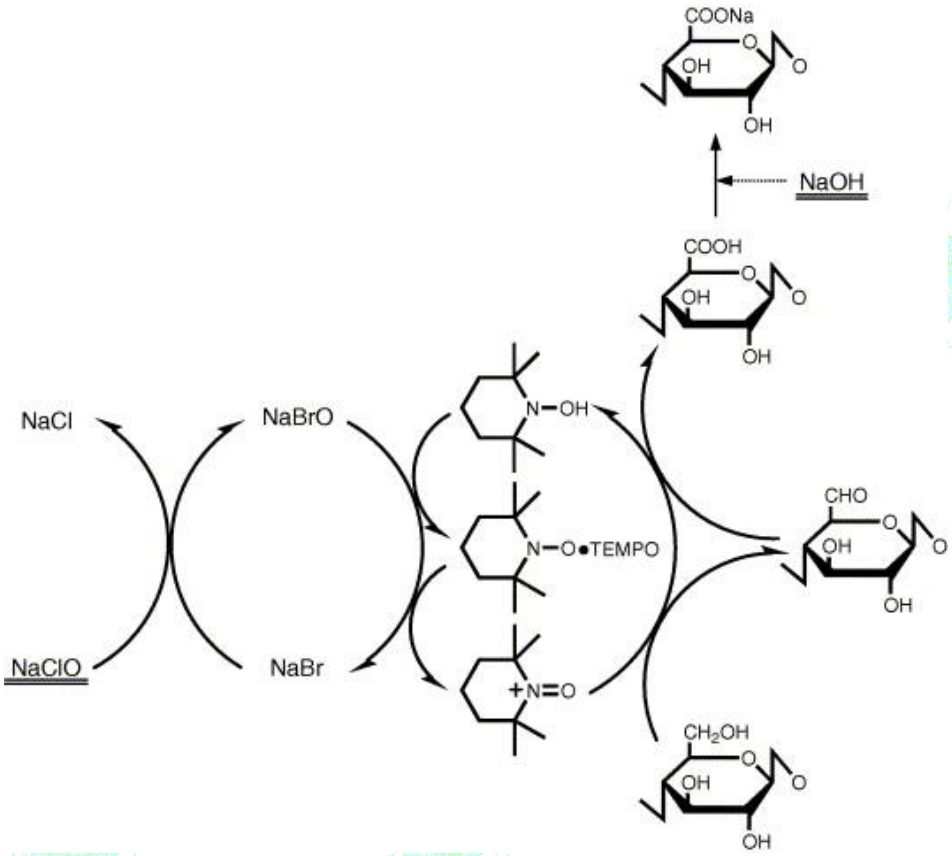

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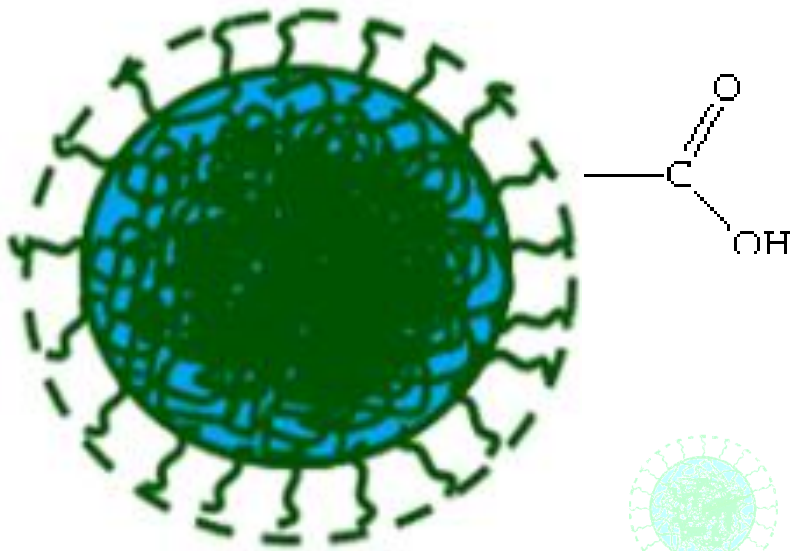
Carbodiimide Cross-linking

EDC



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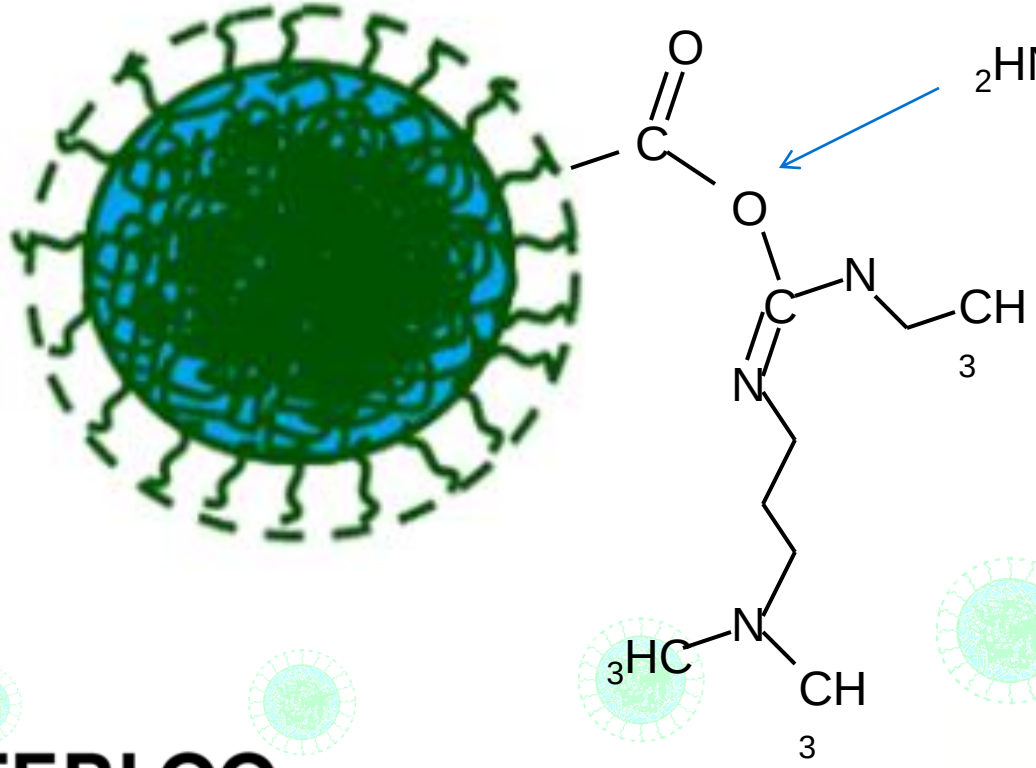

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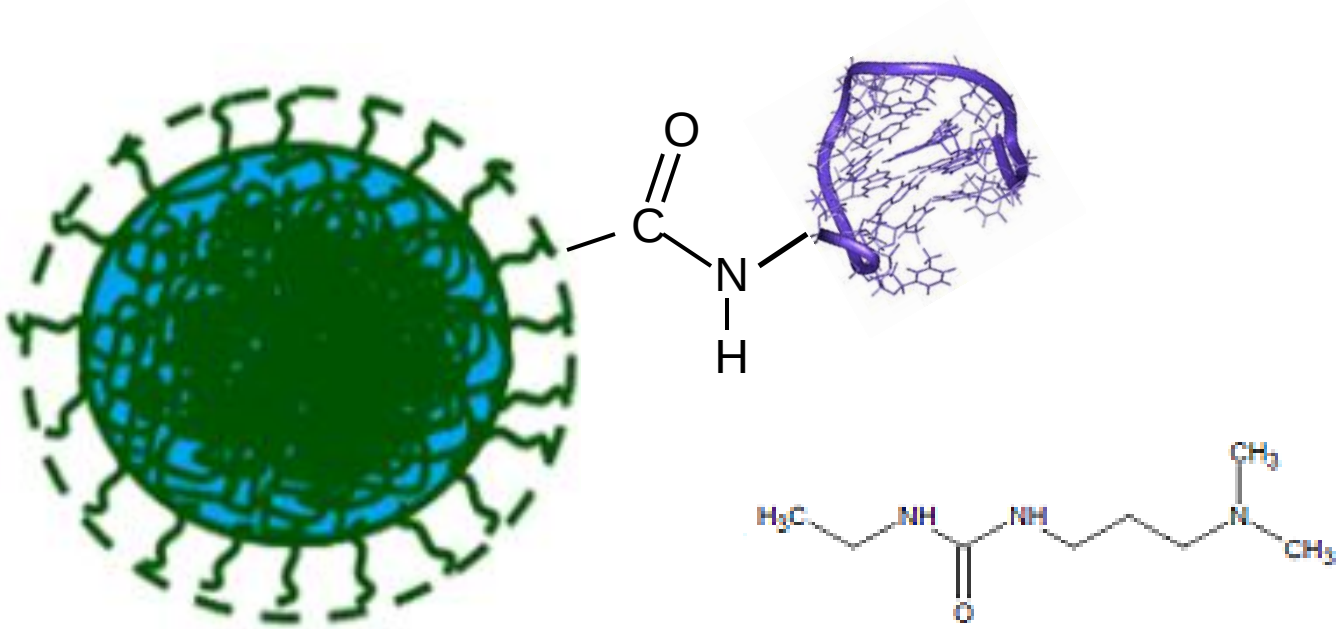

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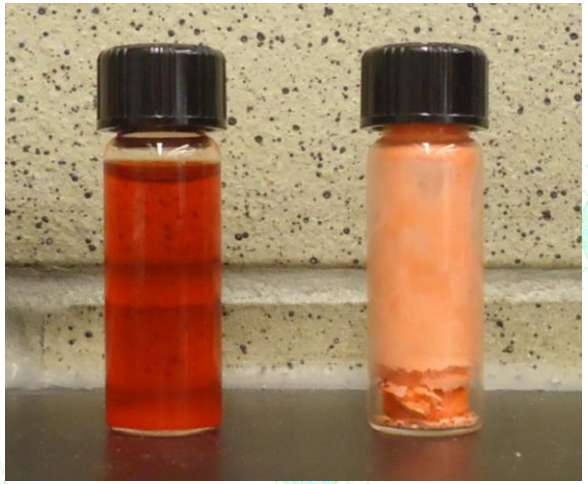
Results

Characterization of Device

	Unmodified EcoSphere	Carboxylated EcoSphere	Aptamer Bioconjugate
Size (nm)	169 ± 15.2	141.2 ± 11.2	156.2 ± 32.4
Zeta Potential (mV)	4 ± 2.3	-25 ± 0.8	-31 ± 1.2



* **Size using NanoSight LM-20**
* **Zeta using Brookhaven Zetasizer**

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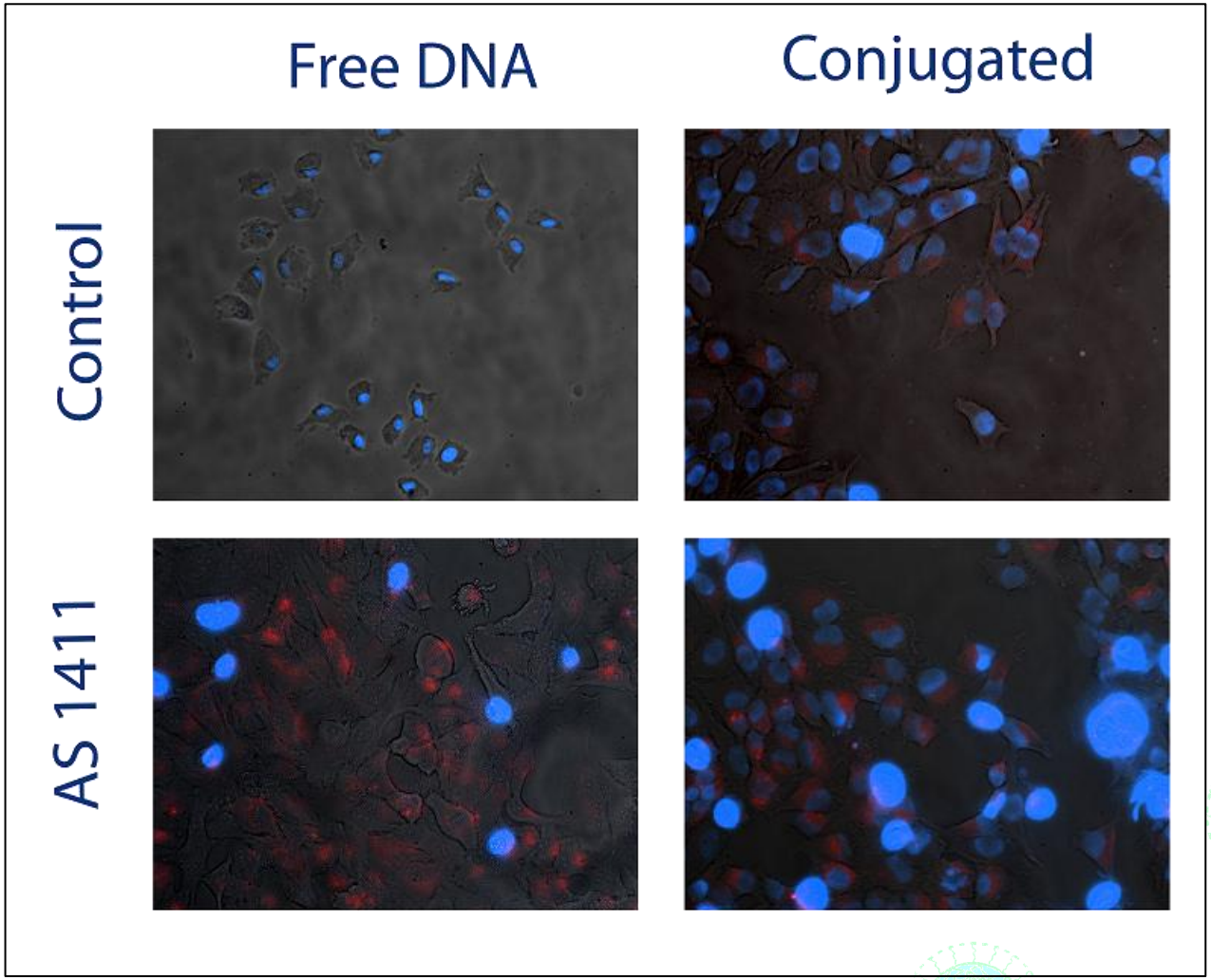

Fluorescence Microscope – HeLa cells, 48 hour incubation
Blue is cell nucleus, red is fluorescent label on DNA/ device

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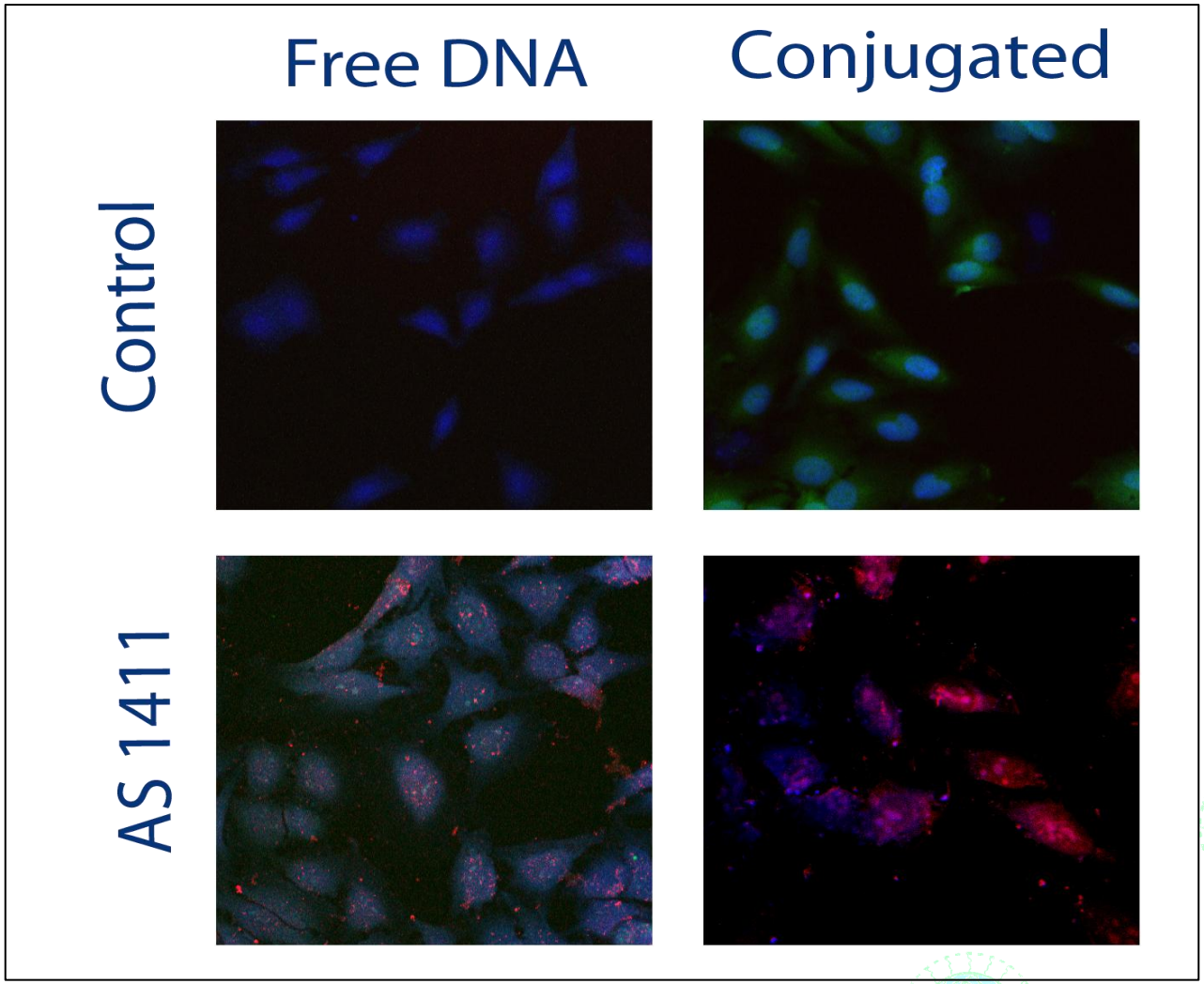

Confocal Microscope Images – HeLa Cells, 2 hour incubation
Blue is cell nucleus/cytoplasm, green is cell cytoplasm, red is DNA/device

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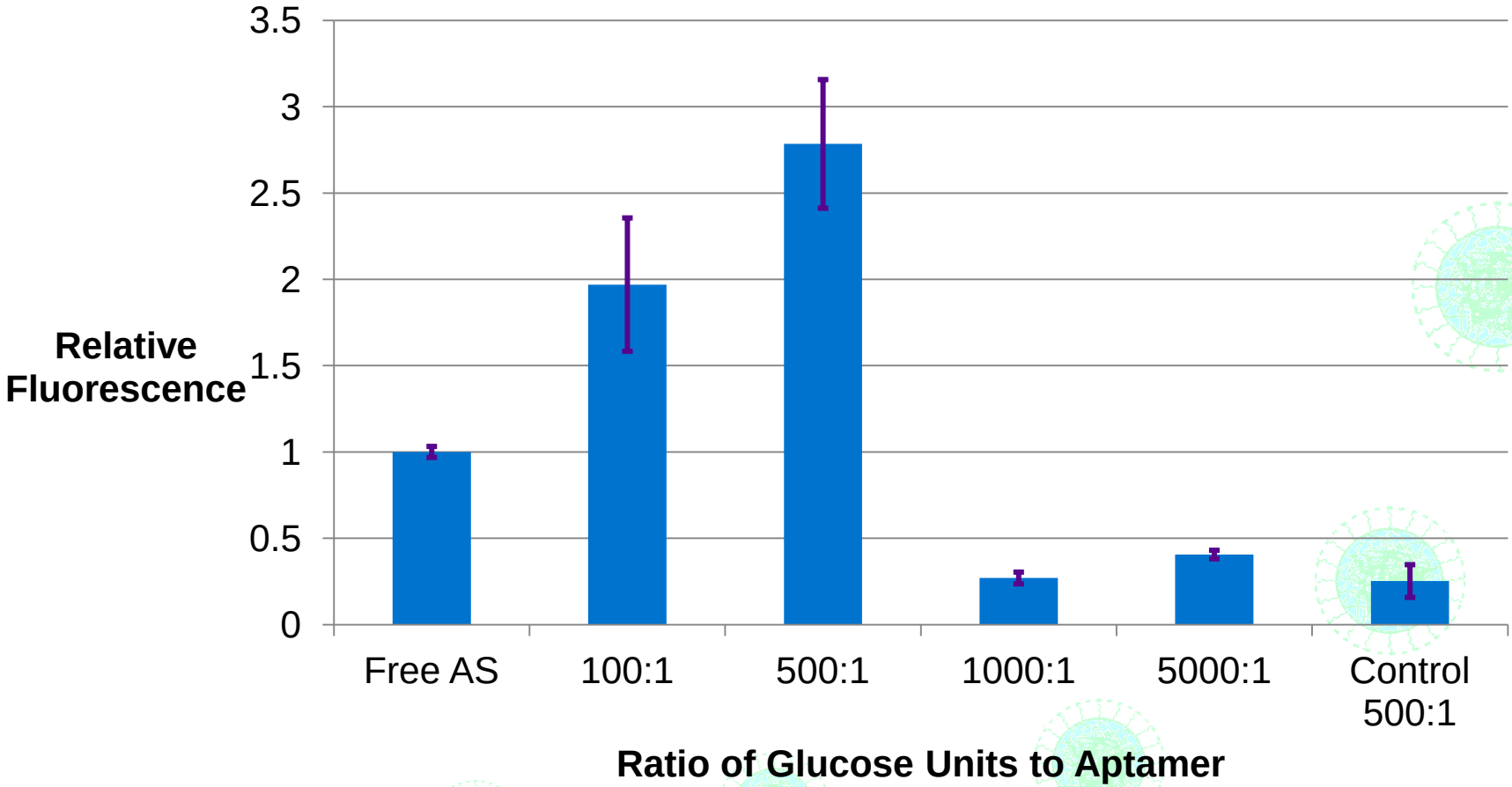
Normalized Cellular Uptake (2 hr. exposure for HeLa cells)



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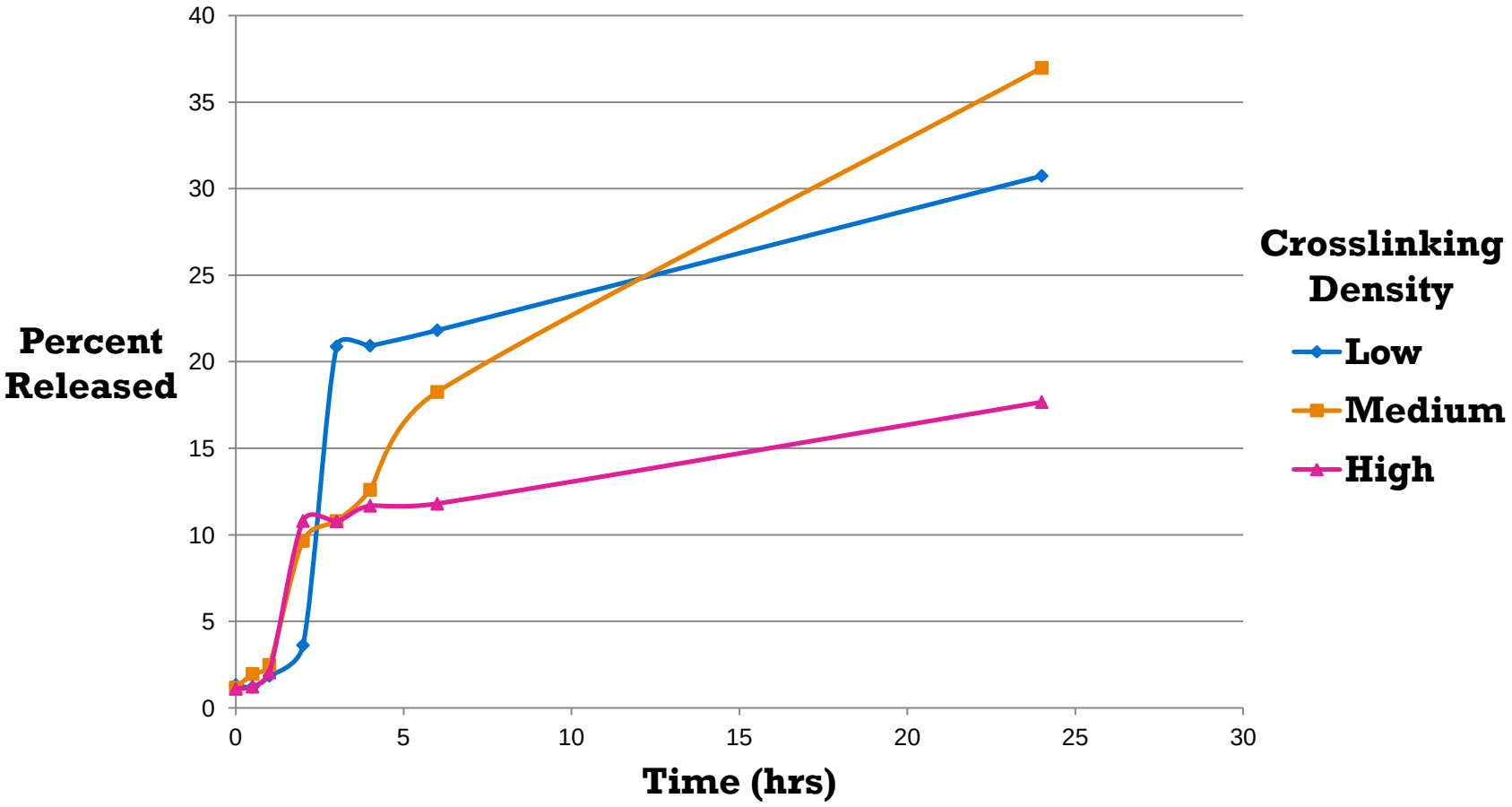
Drug Release Profile



- Doxorubicin release - Using Dialysis tubing of 25 kDa (Doxorubicin = 0.5 kDa)
- DOX measured using ELISA: excitation 470 nm, emission 550 nm

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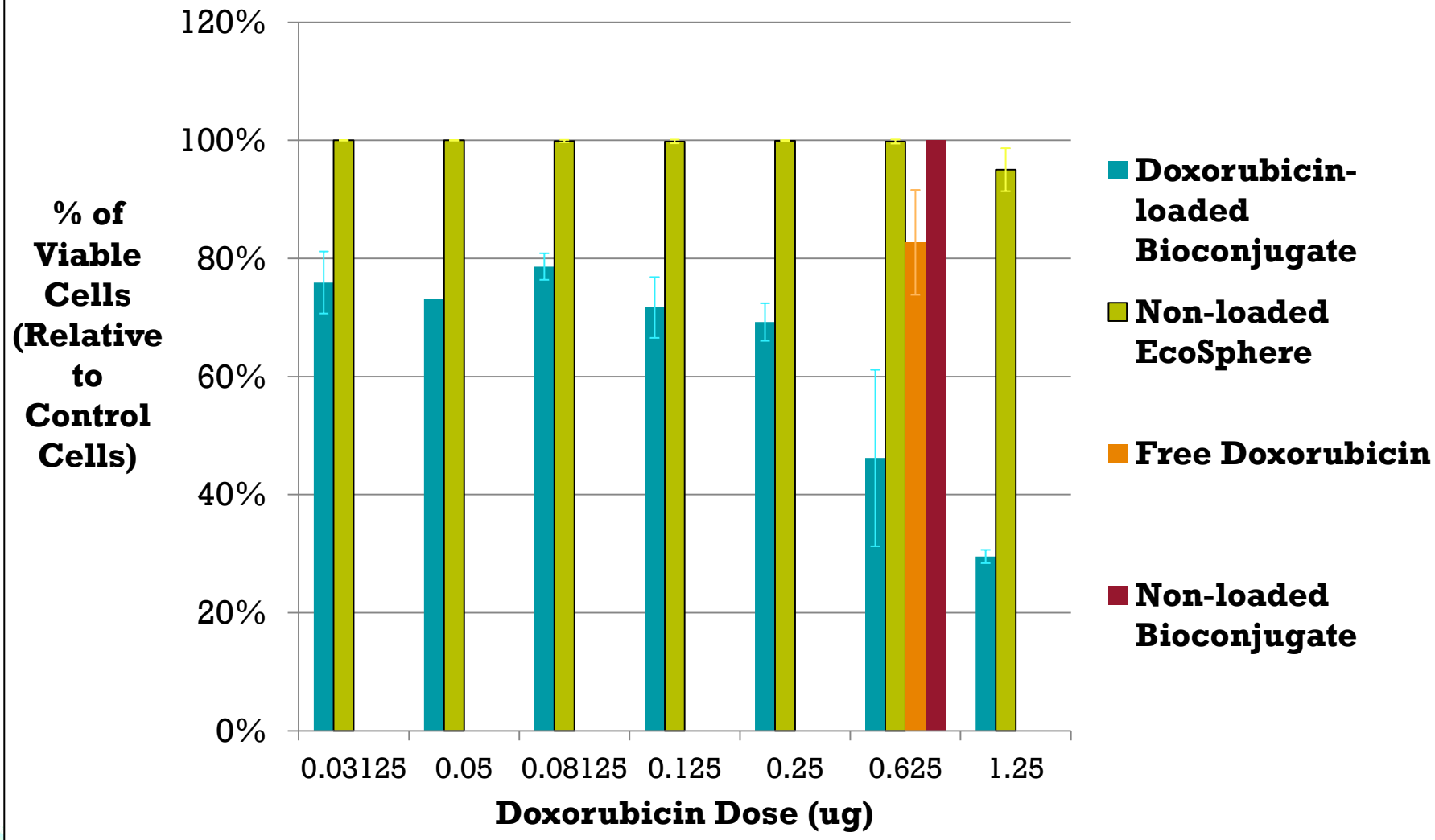
LDH Assay – HeLa Cells (24 hr. exposure)



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Future Work

- Move testing to *in vivo* (animal studies) for further validation of targeting
- Load different therapeutic agents into Ecosphere
- Conjugate to high Z material (TiO_2, Fe) for use as imaging aid/ radiation sensitizer

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Design Checklist:

1. New drug delivery platform using starch nanoparticles
2. Biocompatible and ideal size for “passive” targeting
3. Device uptake by cancer cells
4. Able to load drug and shows sustained release profile
5. Able to kill cancer cells with encapsulated drug better than free drug
6. Can be produced reliably to give similar outcomes

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Questions?

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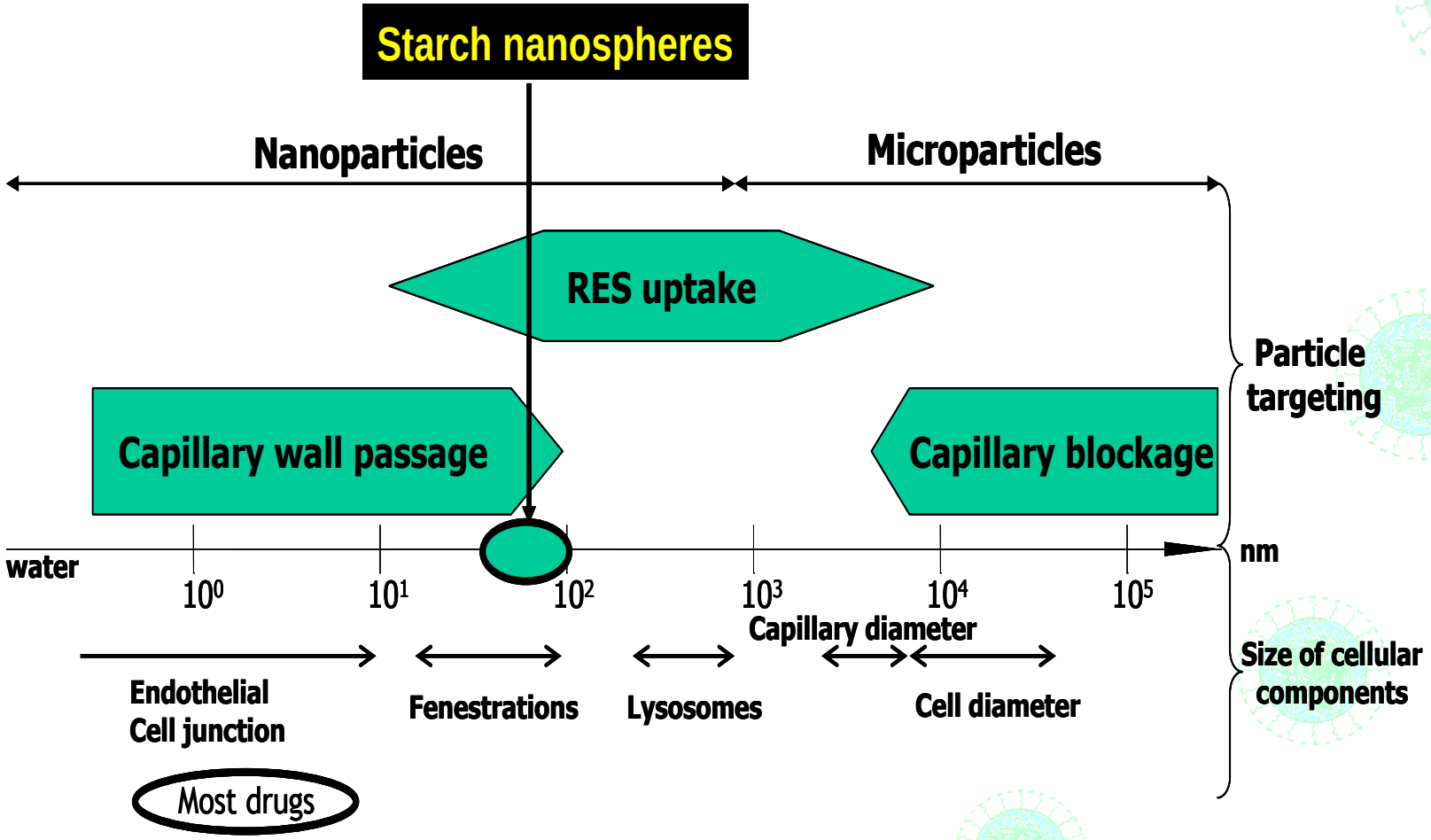

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